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Processing and antibacterial properties of chitosan-coated alginate fibers

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ABSTRACT

The preparation of chitosan-coated alginate fibers by a wet spin process is presented and the characterization of the antibacterial activities of these fibers is discussed. Preformed calcium alginate fibers were passed in chitosan acetate solutions. The coagulation method of the coating consisted in the immersion of fibers in a bath of calcium dihydroxide solution (0.1 M). The antibacterial evaluation was achieved by a CFU (Colony-Forming Units) counting method after 6 h of incubation at 37 °C. The incorporation of chitosan on calcium alginate fibers brings antibacterial activities against *Staphylococcus epidermidis*, *Escherichia coli* and various *Staphylococcus aureus* strains namely MSSA (Methicillin Sensitive *Staphylococcus aureus*), CA-MRSA (Community Associated Methicillin Resistant *Staphylococcus aureus*) and HA-MRSA (Healthcare Associated Methicillin Resistant *Staphylococcus aureus*) which make these chitosan-coated fibers potential candidates for wound dressing materials. Developing a wound dressing with the haemostatic and healing properties of alginate combined with antibacterial properties of chitosan is envisioned for fighting against the infections and more particularly nosocomial diseases.

1. Introduction

An "ideal" compress should maintain a moist environment at the interface with the wound, allow gas (H_2O) exchange, act as a barrier to microorganisms and should absorb the excess of exudates. It must also be non-toxic, non-allergenic and easy to remove without causing any additional lesion or pain. Polysaccharide biopolymers such as alginates (Orive et al., 2002) and chitosan (Howling et al., 2001; Ladet, David, & Domard, 2008; Ladet, Tahiri, Montembault, Domard, & Corvol, 2011; Malaise et al., 2014; Montembault et al., 2006; Nandi, Kundu, & Basu, 2013; Rami et al., 2014) possess several properties including biodegradability, biocompatibility, low toxicity and low immunogenicity. Only few exhibit an antimicrobial activity such as chitosans (El-Tahlawy & Hudson, 2006; Knill et al., 2004; Kong et al., 2010; L. Wang, Khor, Wee, & Lim, 2002). For these reasons, it was considered to develop a multicomponent wound dressing made of biocompatible materials promoting healing while having antimicrobial properties and requiring a minimum number of processing steps and thus acceptable manufacturing costs (Boateng, Matthews, Stevens, & Eccleston, 2008; Jarry et al., 2001; Kara, Aksoy, Yuksekdag, Hasirci, & Aksoy, 2014; Mi et al., 2001; Shin, Yoo, & Min, 1999; Strand, Vandvik, Varum, & Ostgaard, 2001).

Alginates are polyanionic, linear, binary copolymers of β-(1–4)-linked D-mannuronic acid (M) and its C-5 epimer α-(1–4)-linked L-guluronic acid (G) (Draget, Østgaard, & Smidsrød, 1990). The structure of alginates is characterized by the global molar ratio of G/M residues, but also the fraction and average lengths of G-blocks (GGG), M-blocks (MMM) and alternated blocks of M and G (MGMG) (Harding, Vårum, Stokke, & Smidsrød, 1991). Alginates have the ability to gel via ionotropic gelation or neutralization. The latter mechanism occurs at pH values below the pK_a of alginate (acid gelation) (Draget, Skjåk Bræk, & Smidsrød, 1994). The pK_a of alginates lies between 3.4 (pK_a of mannuronic residues within M blocks) and 3.65 (pK_a of guluronic residues within G blocks) (Agulhon, Robitzer, Habas, & Quignard, 2014; Haug, Larsen, & Smidsrød, 1967).

Chitosan is the main biopolymer derived from chitin. This natural polymer consist in D-glucosamine and N-acetyl-D-glucosamine linked by β(1→4) glycosidic linkages. The physico-chemical properties of chitosans are largely impacted by its chemical structure (average molecular weights), degree of acetylation (DA or molar fraction of N-

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acetyl-D-glucosamine residues) (Schatz, Viton, Delair, Pichot, & Domard, 2003) but also the sequence of acetylated and non-acetylated residues along the chain (Roberts, 1992). The physical gelation of chitosans will depend on such molecular structure parameters (Cataldo, Crea, Gianguzza, Pettignano, & Piazzese, 2009), but also on the physico-chemical context of gelation. Moore and Roberts observed the formation of non-reversible gels by chitosan reacetylation (Moore & Roberts, 1980a, 1980b). Vachoud et al. have observed an increase of the gelling speed by chitosan reacetylation with the molecular weight of polymers (Vachoud, Zydowicz, & Domard, 1997). Montembault et al. extensively studied the gelation of chitosan solutions in contact with a base, when the pH is increased well above the pK_a of amine moieties (ranging between 6.2 to 6.5 according to the DA) (Montembault, Viton, & Domard, 2005). Chitosans exhibit antibacterial activity on both Gram-positive bacteria (e.g.: *Staphylococcus aureus*, *Pseudomonas aeruginosa*) and Gram-negative bacteria (e.g.: *Escherichia coli*) (Jarry et al., 2001; Mi et al., 2001; Shin et al., 1999; Strand et al., 2001). Several authors have shown an antibacterial activity of chitosan derivatives against MRSA (Methicillin Resistant *Staphylococcus aureus*) (ex: gallic acid-grafted-chitosan (Lee et al., 2014), N-quaternary chitosans (Rúnarsson et al., 2010)). Complex nanoparticle formulations containing chitosan were also tested (Banche et al., 2015). These results do not fully predict the properties of unmodified and pure chitosan-based materials or coatings.

Other studies use chitosan as a drug delivery system to deliver antiseptics and antibiotics against MRSA (Cevher et al., 2006; Smith et al., 2013), but they rarely focus on the intrinsic properties of chitosan.

A recent investigation (Costa, Silva, Tavaria, & Pintado, 2017) is devoted to the intrinsic antibiofilm activity against MRSA. Chitosan is shown to be a potential bioactive agent for the control of biofilm formation related to MRSA infections. In this latter work, acidic aqueous solutions of chitosan acetate were studied. Thus, the anti-bacterial effect possibly resulted both from chitosan bioactivity and the acid used to prepare the solution. In the present work, the antibacterial activity of unmodified pure chitosan coatings was studied (in the solid state, after washing the salts), for different bacterial strains and well characterized chitosans.

Considering the electrostatic association of chitosans and alginates, several authors exploited the polyelectrolyte complexation occurring when both polyelectrolytes are charged. Thus, polyelectrolyte complex (PEC) can be obtained in the form of colloids, layer-by-layer assembly (Zou & Kim, 2012) or macroscopic gels (Costalat, Alcouffe, David, & Delair, 2015). Fiber spinning by polyelectrolyte complexation is possible but it is a subtle process to control since PEC formation is impacted by a variety of physico-chemical parameters (molecular weight of the polyelectrolytes, density of charge, amount and nature of the counterions and added salts, nature of the solvents) and even the association protocol (Costalat et al., 2015) may play a role in the complexation. Different methods have been explored to make woven or non-woven textiles from alginate/chitosan fibers. The first is to separately make the alginate and chitosan fibers and then associate them in the same non-woven fabric (Stęplewski, Wawro, Niekraszewicz, & Ciechańska, 2006). In order to avoid making two parallel spinning processes, a challenge is to produce alginate/chitosan mixed fibers in the same spinning process. In this way, electrospun or co-electrospun alginate/chitosan composite fibers were obtained by Chang, Lee, Wu, Yang, and Chien (2012a, 2012b), Hu and Yu (2013) or Jeong et al. (2011). Nevertheless, the electro-spinning is mainly used to develop scaffold structures. To develop multicomponent wound dressings, the wet-spinning method is more adapted. Tamura, Tsuruta, and Tokura (2002) and Sweeney, Miraftab, and Collyer (2014) formed core-sheath alginate/chitosan fibers by extruding sodium alginate solution in a bath containing an aqueous calcium chloride chitosan solution. Nevertheless, high chitosan concentrations and/or high molecular weight chitosans tend to precipitate in the presence of calcium ions. This technique is restricted to small molecular weight chitosans or low chitosan concentrations, leading to a poor incorporation of chitosan on alginate fibers. Sibaja et al. (2015) used an acidic chitosan solution (at pH < 3) as coagulating bath to induce an acid coagulation of alginate and to coat the alginate fibers with chitosan at the same time by polyelectrolyte complexation. This technique requires an excess of acetic acid causing partial hydrolysis of chitosan and alginate, and may induce a drift in their physico-chemical properties from the beginning to the end of the spinning process if the coagulation bath is not continuously regenerated. In the context of industrial development, where spinning may be carried out over several hours, such an evolution of the manufactured fibers is not advantageous. Watthanaphanit and Tamura et al. produced novel alginates/chitosans fibers by injecting a sodium alginate solution containing chitosan whiskers in a first coagulating bath (5 % w/v $CaCl_2$ in 50% v/v MeOH aqueous solution) and a second coagulating bath (MeOH). MeOH is a non-solvent for alginate, therefore it helps stabilize and dry the fibers (Watthanaphanit, Supaphol, Tamura, Tokura, & Rujiravanit, 2010). The bioactivity of chitosan whiskers embedded in the alginate matrix should be expected to be different from a chitosan coating around alginate fibers. Nevertheless, the complex manufacture and cost of chitosan whiskers must be taken into account in sustainable industrial development.

In this work, we developed a chitosan coating process of alginate fiber that is transposable on an industrial scale. Furthermore, we have evidenced that the mechanical, physico-chemical and anti-bacterial properties of resulting fibers were compatible to the context of the preparation of second generation compresses for wound healing.

2. Experimental

2.1. Materials

2.1.1. Sodium alginate

Sodium alginate is provided from FMC industry. It is extracted from brown seaweed, *Laminaria hyperborean*. It is used industrially for the preparation of different medical devices.

2.1.2. Chitosan purification

Chitosan was purchased from Mahtani Chitosan (batch number type 244, obtained from shrimp shells). To obtain a high-purity material, chitosan was dissolved in water at 1% (w/v) in presence of the stoichiometric amount of acetic acid respectively to $–NH_2$ moieties. After complete dissolution, it was filtered successively on 3, 0.8 and 0.45 µm membranes (Millipore). Then, an ammonia solution was added in order to fully precipitate the polymer. The precipitate was repeatedly rinsed with deionised water and centrifuged, until a neutral pH was achieved. Finally, the precipitate was lyophilized.

2.1.3. Reduction of chitosan's molecular weight

Reduction of chitosan's molecular weight was performed by a nitrous deamination (Allan & Peyron, 1995a, 1995b). Chitosan was dissolved at room temperature in 0.15 M ammonium acetate/0.2 M acetic acid buffer (pH = 4.5) at 0.5 % (w/v). After complete dissolution, a sodium nitrite solution at 10 g/L was added corresponding to the molar ratio $r = (n_{NaNO2}/n_{GlcN}) = 0.1$. Mechanical stirring at room temperature was applied during 1 h and then an ammonia solution was added to stop the reaction. The resulting precipitate was repeatedly rinsed with deionised water, centrifuged until a neutral pH was achieved, and lyophilized.

2.1.4. Chitosan and alginate characterizations

2.1.4.1. ^1H nuclear magnetic resonance spectroscopy. The degree of acetylation (DA) of chitosan was calculated from ^1H NMR spectroscopy. The sample of chitosan (15 mg) was dissolved in D_2O (2 mL) with HCl (10 µL). Spectra were recorded on a Bruker Avance III 400 spectrometer (400 MHz) at 25 °C. As proposed by Hirai, Odani, and Nakajima (1991), the DA was deduced from the ratio of the integrated